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## (54) Extract from Ginkgo biloba leaves, its method of preparation and pharmaceuticals containing the extract.

(57) The invention relates to an improved extract from Ginkgo biloba leaves, a method of preparation of the same and pharmaceuticals containing the extract.

EP 0 431 536 A1

# EXTRACT FROM GINKGO BILOBA LEAVES, ITS METHOD OF PREPARATION AND PHARMACEUTICALS CONTAINING THE EXTRACT

The invention relates to an improved extract from Ginkgo biloba leaves, a method of preparation of the extract and the pharmaceuticals containing the extract.

Extracts from the leaves of Ginkgo biloba have been used for a long time for the therapy of peripheral and cerebral arterial circulatory disturbances. Methods of preparation of Ginkgo biloba extracts with a greatly enriched content of flavone glycosides as the active components are known; see DE-B 17 67 098 and DE-B 21 17 429. These extracts are also referred to as Ginkgo biloba monoextracts.

EP-A 0 324 197 describes a method of preparation of an extract from Ginkgo biloba leaves in which an aqueous solution of a lower alcohol or ketone, obtained after extraction of the leaves, is concentrated in the presence of kieselguhr. The resultant aqueous suspension is filtered through kieselguhr, the fitrate is extracted with butanone and the extract is freed from the solvent.

EP-A 330 567 relates to a method of preparation of an extract from Ginkgo biloba leaves in which the crushed leaves are extracted with an aqueous ketone compound. This extract is concentrated until biflavones and hydrophobic compounds precipitate. After filtration the aqueous concentrate is rendered alkaline, whereby the proanthocyanidins precipitate. After separation of the precipitate and acidification of the filtrate, a liquid-liquid-extraction is carried out with a $C_{4-6}$-ketone compound in the presence of ammonium sulfate. The extract is obtained after stripping of the ketone compound.

DE-A 35 14 054 has disclosed that the ginkgolides, known components of the Ginkgo biloba leaves which are terpenoid substances with lactone structure (see K. Nakanishi, Pure and

Applied Chemistry, Vol. 14 (1967), 89-113, and M. Maruyama et al., Tetrahedron Letters (1967), 299-302 and 303-319, and K. Okabe et al., J. Chem. Soc. (1967), 2201-2206), can be used to treat illnesses and similar conditions caused by PAF ("Platelet Activating Factor").

The use of bilobalide, a further substance contained in the Ginkgo biloba leaves, is known from DE-A 33 38 995 and the corresponding US-A 4 571 407 for the treatment of demyelinating neuropathies, encephalopathies and cerebral edemas. Bilobalide is a sesquiterpene lactone structurally related to ginkgolides (see K. Nakanishi et al., R. T. Major et al., and K. Weinges et al., J. Am. Chem. Soc., Vol. 93 (1971), 3544-3546).

Besides the compounds mentioned above, Ginkgo biloba leaves also contain so-called ginkgolic acids (anacardic acids). These compounds are 6-alkylsalicylic acids with n-$C_{13}$- to n-$C_{19}$-alkyl groups with 0 to 3 double bonds; see J. L. Gellermann et al., Phytochemistry, Vol. 15 (1976), 1959-1961 and Analytic. Chem., Vol. 40 (1968), 739-743.

"Ginkgol", a phenol substituted with the corresponding alkyl group, can be obtained either biogenetically by decarboxylation of the ginkgolic acids or during the technical processing of the Ginkgo biloba leaves; see Kawamura, Japan, J. Chem., Vol. 3 (1928), 91-93.

The ginkgolic acids and ginkgols in Ginkgo biloba are accompanied by corresponding derivatives with a further phenolic hydroxyl group in 4-position, the 6-alkylresorcylic acids or 5-alkylresorcins; see J. Gellermann et al., Phytochemistry, Vol. 15 (1976), 1959-1961. These resorcin derivatives are responsible for the toxic effects and especially for the strong allergies and contact dermatitis caused by toxicodendron plants; see G. A. Hill et al., J. Am. Chem. Soc., Vol. 56 (1934), 2736-2738.

Cases of strong allergic reactions after contact with Ginkgo fruits are known; see W.F. Sowers et al., Arch. Dermatol., Vol. 91 (1965), 452-456, and T. Nakamura, Contact Dermatitis, Vol. 12 (1985), 281-282. Serious mucosal disturbances after eating Ginkgo fruits have been described; see L. E. Becker and G. B. Skipworth, J. Am. Med. Assoc., Vol. 231 (1975), 1162-1163. Allergic skin reactions also occur occasionally on collecting or handling Ginkgo leaves.

The significance of allergies caused by alkylphenol compounds from anacardiaceae and ginkgoaceae is evident from the development of substances and methods of desensitisation described in patent literature (see US-A 4 428 965) against the allergies caused by alkylphenol compounds.

Commercial extracts from Ginkgo biloba leaves contain between 50 and 10,000 ppm ginkgolic acids.

The extracts from Ginkgo biloba leaves prepared by the known methods in DE-B 17 67 098 and DE-B 21 17 429 are substantially free of alkylphenol compounds because the lipophilic components of the extract are removed by a liquid-liquid-extraction of the aqueous acetone extract with a substantially water-immiscible lipophilic solvent, e.g. with a chlorinated aliphatic lower hydrocarbon such as carbon tetrachloride. However, in this step, the therapeutically valuable ginkgolides and the bilobalide are also considerably reduced so that their content in the final product in Example 1 of DE-B 21 17 429 is a maximum of 0.5% in the case of ginkgolides A, B, C and J in total and approximately 0.3% in the case of bilobalide. The quantity

of flavone glycosides, however, is greatly increased during this step, namely from 3 to 4% in the crude extract to approximately 24% in the final product.

The object of the present invention therefore is to provide an extract from Ginkgo biloba leaves which is substantially free of alkylphenol compounds, has a high content of flavone glycosides and which contains substantially all of the ginkgolides and bilobalide present in the leaves used.

A further object of the invention is to provide a method of preparation of the extract from Ginkgo biloba leaves which is substantially free of alkylphenol compounds and which has a high content of flavone glycosides, ginkgolides and bilobalide. The method of the present invention should, in contrast to the known methods in DE-B 17 67 098 and DE-B 21 17 429, succeed in removing the alkylphenol compounds without the use of chlorinated aliphatic hydrocarbons. The use of chlorinated hydrocarbons in technical processes is very problematic because of the occupational medical risks, the potential danger of these compounds to the environment and the possibility of undesirable residues in pharmaceuticals.

A further advantage of the method of the present invention compared to the method in DE-B 21 17 429 is that no lead compounds are used in the removal of the undesirable polyphenol compounds with tanning properties (proantho cyanidins). Compounds of lead are most undesirable because of the health risks for the people involved, and over and above that, the costs involved for their proper disposal are considerable.

Finally, it is the object of the invention to provide pharmaceuticals which contain this Ginkgo biloba extract with a high content of flavone glycosides, ginkgolides and bilobalide and where there is substantially no danger of allergic reactions, precisely because of the removal of the alkylphenol compounds.

The invention therefore relates to an extract from Ginkgo biloba leaves which is substantially free of alkylphenol compounds, which has a high flavone glycoside content and which contains most of the ginkgolides and the bilobalide originally present in the leaves. Preferably the extract in the present invention should contain

- 20 to 30 weight percent, in particular 22 to 26 weight percent, flavone glycosides,
- 2.5 to 4.5 weight percent of ginkgolides A, B, C and J (in total),
- 2.0 to 4.0 weight percent bilobalide,
- less than 10 ppm, in particular less than 1 ppm, alkylphenol compounds and
- less than 10 weight percent proanthocyanidins.

In addition, the invention relates to a method of preparation of this Ginkgo biloba extract from Ginkgo biloba leaves which comprises the steps described in Claims 3 - 6. In contrast to the method of separating the lipophilic components described in DE-B 17 67 098, the aqueous alcohol or aqueous acetone crude extract is not directly subjected to liquid-liquid-extraction with a chlorinated aliphatic hydrocarbon, but rather most of the lipophilic components, which precipitate on distillation of the organic solvent components and dilution with water to a maximum content of 10 weight percent, preferably 5 weight percent, are separated by filtration. The alkylphenol compounds, the chlorophyll, the fatty acid derivatives and the biflavones precipitate due to their low solubility in water and can be separated by filtration. Under these conditions, the desired components of the Ginkgo biloba extract remain dissolved.

Subsequently, the methylethylketone or methylethylketone/ acetone-extracts are prepared according to DE-B 17 67 098 and DE-B 21 17 429. In contrast to the method in DE-B 21 17 429, however, a lead compound or a polyamide is not used to reduce the content of proanthocyanidins to less than 10%, but rather a distribution of the butanone extract is carried out between water and a water-immiscible $C_{4-5}$-alkanol, whereby the proanthocyanidins remain in the water phase.

In a preferred embodiment the extraction with methylethylketone or methylethylketone/acetone is directly replaced by extracting the aqueous extract solution freed from the lipophilic components with a water-immiscible alkanol of 4 or 5 C-atoms. For economic reasons n-butanol is preferred. 10 to 30 weight percent of sodium chloride or ammonium sulfate can be added to the aqueous extract solution. The alkylphenol compounds are reduced further to a content of less than 10 ppm in a subsequent decreasing step by removing the solvent from the butanol or pentanol extract by distillation, preparing a solution with 5 to 20 weight percent solids content in 20 to 60 weight percent of aqueous ethanol and subjecting this solution to a multistep liquid-liquid-extraction with an aliphatic hydrocarbon with a boiling point of 60 to 100° C.

In addition, the invention relates to pharmaceuticals which according to Claim 7 are characterized by a content of Ginkgo biloba extract.

In pharmacological experimental models, the extract prepared according to the present invention has radical scavenging properties and properties which stimulate the circulation of blood, prevent ischemic disorders and inhibit platelet aggregations.

The Ginkgo biloba extract of the invention can be processed in the usual way for the preparation of pharmaceuticals e.g. to solutions, coated tablets, tablets or injection preparations. The pharmaceuticals in

the invention are used for the treatment of peripheral and cerebral arterial circulatory disturbances.

The examples illustrate the invention. Parts and percentage data refer to weight unless otherwise stated.

Example 1

100 kg of dry Ginkgo biloba leaves are crushed in a mill to a particle size of less than 4 mm. After adding 750 kg of 60 weight percent aqueous acetone the mixture is stirred intensively for 30 minutes at a temperature of 57 to 59 °C. The solid residue is separated by filtration or centrifugation and subjected to a second extraction under the same conditions. The extracts from the first and second extraction steps are combined. The ginkgolic acid content (based on the dry extract) equals approximately 13,000 ppm. The resultant extract is concentrated under reduced pressure to a solids content of 30 to 40% and a maximum of approximately 5 weight percent acetone. By adding water, the concentrate is diluted to double volume and, while being stirred, left to cool to approximately 12 °C. A precipitate forms which contains most of the ginkgolic acids, that is, the alkylphenol compounds, present in the leaves. After one hour at this temperature, the resultant precipitate is separated by centrifugation and discarded.

The ginkgolic acid content in the resultant aqueous supernatant (based on the dry extract) equals approximately 320 ppm.

30 parts of ammonium sulfate are added to 100 parts of the aqueous solution. The mixture is stirred. After the ammonium sulfate has dissolved, a liquid-liquid-extraction is carried out twice with a mixture of methylethylketone and acetone in a ratio of 6 : 4 to 1 : 1, whereby the organic solvent added is equivalent to half the volume of the aqueous solution and, after intensive stirring and pumping, the organic upper phase formed on completion of the mixing process is removed.

The methylethylketone acetone solution is then concentrated under reduced pressure to a solids content of 50 to 70%. This concentrate is diluted with water to a solids content of 10%.

This substantially aqueous extract solution is stirred three times, each time with half of its volume of water-saturated n-butanol. The combined butanol phases are concentrated under reduced pressure to a solids content of at least 50%. To remove the n-butanol from the highly concentrated extract by azeotropic distillation, water, preferably, is added. The resultant aqueous concentrate is diluted with water and ethanol so that a solution with 10 weight percent dry extract in 30 weight percent aqueous ethanol is obtained.

To reduce the alkylphenol compounds to a residual content of less than 10 ppm, this solution is stirred at least three times at room temperature, each time with 1/3 of its volume of n-heptane.

The water phase is concentrated under reduced pressure to a solids content of at least 50% and dried at a maximum product temperature of approximately 60 to 80 °C to a dry extract with a water content of less than 5%.

From 100 kg of Ginkgo leaves, 2.7 kg of Ginkgo biloba extract with a content of 24.8 weight percent flavone glycosides, 3.2% ginkgolides, 2.9% bilobalide, approximately 5% proantho cyanidins and less than 1 ppm alkylphenol compounds are obtained.

Example 2

The aqueous extract solution obtained in Example 1, following separation by centrifugation of the precipitate consisting predominantly of lipophilic components, is stirred three times, each time with half of its volume of butan-2-ol (sec. butylalcohol).

The resultant butan-2-ol solution is evaporated under reduced pressure until a concentrate with at least 50% solids content is obtained. Preferably water is added to remove the butanol from the highly concentrated extract by azeotropic distillation. Following dilution with water and ethanol to a solids content of approx. 10% and approx. 30 percent by weight ethanol in the solution, the solution is stirred three times, each time with 1/3 of its volume of cyclohexane.

The water phase is concentrated under reduced pressure to a solids content of at least 50% and dried at a maximum temperature of 60 to 80 °C to a dry extract with a water content of less than 5%.

From 100 kg of Ginkgo leaves, 2.9 kg of Ginkgo biloba extract with a content of 25.3% flavone glycosides, 3.4% ginkgolides, 3.1% bilobalide, approximately 4.2% proanthocyanidins and less than 1 ppm alkylphenol compounds are obtained.

Example 3

Solution for oral administration:
100 ml solution contains:

| | |
|---|---|
| Ginkgo biloba extract | 4.0 g |
| ethanol | 50.0 g |
| demineralised water to | 100.0 ml |

Example 4

Coated tablets:
1 tablet contains:

| | | |
|---|---|---|
| Ginkgo biloba extract | | 40.00 mg |
| microcrystalline cellulose | | 100.00 mg |
| lactose | | 80.00 mg |
| colloidal silicic acid | | 25.00 mg |
| talcum (in core) | | 4.50 mg |
| magnesium stearate | | 0.50 mg |
| hydroxypropyl methylcellulose | | 12.00 mg |
| ferric oxide pigment | | 0.10 mg |
| talcum (in coat) | | 0.50 mg |
| weight of a coated tablet | approx. | 262.60 mg |

## Claims

1. Extract from the leaves of Ginkgo biloba which is substantially free of alkylphenol compounds and has an increased content of flavone glycosides, characterized in that it contains most of the ginkgolides and bilobalide originally present in the leaves.

2. Extract according to Claim 1, containing
   - 20 to 30 weight percent, in particular 22 to 26 weight percent, flavone glycosides,
   - 2.5 to 4.5 weight percent of ginkgolides A, B, C and J (in total),
   - 2.0 to 4.0 weight percent bilobalide,
   - less than 10 ppm, in particular less than 1 ppm, alkylphenol compounds and
   - less than 10 weight percent proanthocyanidins.

3. Method of preparation of an extract from the leaves of Ginkgo biloba which is substantially free of alkylphenol compounds and has an increased content of flavone glycosides and a content of ginkgolides and bilobalide which corresponds to most of these components originally present in the leaves, the method comprising an extraction of the leaves with aqueous acetone, an aqueous alkanol of 1 to 3 C-atoms or anhydrous methanol, a step to remove the lipophilic components, at least one treatment with ammonium sulfate and the subsequent extraction with methylethylketone or a mixture of methylethylketone and acetone, and said method being characterized in that most of the organic solvent is separated from the extract from the leaves containing the aqueous organic solvent, the remaining aqueous solution is diluted to a solids content of 5 to 25 weight percent, preferably approximately 15 to 20 weight percent, and left to cool and stand until a precipitate forms from the lipophilic components which do not dissolve well in water, and then this precipitate is separated, and

that the butanol or pentanol extract prepared directly by extraction of the filtrate, or the aqueous alcohol solution obtained following the distribution of a methyethylketone-acetone-extract between butanol or pentanol and water, is extracted with an aliphatic or cycloaliphatic solvent with a boiling point of approximately 60-100 °C in order to further separate the alkylphenol compounds.

4. Method of preparation of an extract from Ginkgo biloba leaves, containing
   - 20 to 30 weight percent, in particular 22 to 26 weight percent, flavone glycosides,
   - 2.5 to 4.5 weight percent of ginkgolides A, B, C and J (in total),
   - 2.0 to 4.0 weight percent bilobalide,
   - less than 10 ppm, in particular less than 1 ppm alkylphenol compounds and
   - less than 10 weight percent proanthocyanidins and characterized in that

   (a) the fresh or dried green leaves of Ginkgo biloba are extracted at a temperature of approximately 40 to 100 °C with aqueous acetone, an aqueous alkanol of 1 to 3 C-atoms or anhydrous methanol,
   (b) most of the organic solvent is separated from the extract to a maximum content of 10 weight percent, preferably a maximum of 5 weight percent, whereby water can be added in the last steps of distillation,
   (c) the remaining concentrated aqueous solution is diluted with water to a solids content of 5 to 25 weight percent, preferably 15 to 20 weight percent, left to cool, while being stirred, to a temperature below 25 °C, preferably approximately 10 to 12 °C, left to stand until a precipitate forms and the resultant precipitate consisting of the lipophilic components which do not dissolve well in water is removed,
   (d) ammonium sulfate is added to the remaining aqueous solution to give a content of 30 weight percent and the solution formed is extracted with methylethylketone or a mixture containing methylethylketone and acetone in a ratio of 9 : 1 to 4 : 6, preferably 6 : 4,
   (e) the extract obtained is concentrated to a solids content of 50 to 70% and the resultant concentrate is diluted with water to a solids content of 5 - 20 %,
   (f) the resultant solution is subjected to a multistep extraction with a water-immiscible butanol or pentanol,
   (g) the butanol or pentanol layers are concentrated to a solids content of 50 - 70%,
   (h) the concentrate is diluted with sufficient water and ethanol to obtain a solution of 5 to 20 weight percent dry extract in 20 to 60 weight percent aqueous ethanol,
   (i) the aqueous alcohol solution is extracted with an aliphatic or cycloaliphatic solvent with a boiling point of approximately 60 to 100 °C in order to further remove the alkylphenol compounds,
   (j) the water phase is concentrated under reduced pressure and dried at a maximum temperature of 60 to 80 °C to a dry extract with a water content of less than 5%.

5. Method according to Claim 4, characterized in that method steps (d) and (e) are left out and the aqueous solution obtained in (c) is processed further according to steps (f) to (j), but that in step (f), 10 to 30 weight percent of sodium chloride or ammonium sulfate, preferably 20% of ammonium sulfate can be added to the aqueous solution.

6. Method according to any of Claims 3 to 5, characterized in that n-butanol is used in step (f).

7. Pharmaceuticals, characterized by a content of Ginkgo biloba extract according to Claim 1 or 2 or obtainable according to any of Claims 3 to 6.

European Patent Office

# EUROPEAN SEARCH REPORT

Application Number

EP 90 12 3142

## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.5) |
|---|---|---|---|
| A | EP-A-0 086 315 (PRODIPHARM S.A.) | | A 61 K 35/78 |

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

A 61 K

The present search report has been drawn up for all claims

| Place of search | Date of completion of search | Examiner |
|---|---|---|
| The Hague | 07 March 91 | REMPP G.L.E. |

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same catagory
A: technological background
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P: intermediate document
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